

WHAT IS CLAIMED IS:

1           1. An isolated infectious recombinant respiratory  
2           syncytial virus (RSV) comprising a RSV genome or antigenome, a  
3           major nucleocapsid (N) protein, a nucleocapsid phosphoprotein  
4           (P), a large polymerase protein (L), and a RNA polymerase  
5           elongation factor, wherein the recombinant RSV has at least two  
6           attenuating mutations, one of the mutations specifying a  
7           temperature-sensitive substitution at amino acid Phe<sub>521</sub>, Gln<sub>831</sub>,  
8           Met<sub>1169</sub>, or Tyr<sub>1321</sub> in the RSV polymerase gene or a temperature-  
9           sensitive nucleotide substitution in the gene-start sequence of  
10          gene M2.

1           2. The RSV of claim 1, having at least three  
2           attenuating mutations.

1           3. The RSV of claim 1, wherein Leu is substituted at  
2           Phe<sub>521</sub> or Gln<sub>831</sub>.

1           4. The RSV of claim 1, wherein Val is substituted at  
2           Met<sub>1169</sub>.

1           5. The RSV of claim 1, wherein Asn is substituted at  
2           Tyr<sub>1321</sub>.

1           6. The RSV of claim 1, wherein at least two of the  
2           mutations are selected from the group consisting of  
3           temperature-sensitive substitutions at Phe<sub>521</sub>, Gln<sub>831</sub>, Met<sub>1169</sub>, and  
4           Tyr<sub>1321</sub>.

1           7. The RSV of claim 6, wherein the  
2           temperature-sensitive substitutions are at Phe<sub>521</sub> and Met<sub>1169</sub>.

1           8. The RSV of 6, wherein the temperature-sensitive  
2           substitutions are at Gln<sub>831</sub> and Tyr<sub>1321</sub>.

1           9. The RSV of claim 1, wherein two mutations occur in  
2 the codon encoding a temperature-sensitive substitution at  
3 Phe<sub>521</sub>, Gln<sub>631</sub>, Met<sub>1169</sub>, or Tyr<sub>1321</sub>.

1           10. The RSV of claim 1, formulated in a dose of 10<sup>3</sup>  
2 to 10<sup>6</sup> PFU of attenuated virus.

1           11. The RSV of claim 1 further comprising a  
2 nucleotide modification specifying a phenotype selected from a  
3 change in growth characteristics, attenuation,  
4 temperature-sensitivity, cold-adaptation, small plaque size,  
5 host range restriction, or a change in immunogenicity.

1           12. The RSV of claim 11, wherein a SH, NS1, NS2 or G  
2 gene is modified.

1           13. The RSV of claim 12, wherein the SH gene is  
2 deleted.

1           14. The RSV of claim 12, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1           15. The RSV of claim 11, wherein said further  
2 nucleotide modification is to a cis-acting regulatory sequence  
3 that is modified or rearranged within the RSV genome or  
4 antigenome.

1           16. The RSV of claim 15, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1           17. The RSV of claim 16, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene.

1           18. The RSV of claim 11, wherein the nucleotide  
2 modification is selected from a termination codon introduced  
3 within a selected gene or a change in sequence, position, or

4 presence of a GS or GE transcription signal relative to a  
5 selected gene.

1 19. The RSV of claim 11, wherein the nucleotide  
2 modification comprises an inserted, deleted or altered  
3 translational start codon in sequence context within the RSV  
4 genome or antigenome.

1 20. The RSV of claim 11, wherein a gene or gene  
2 segment encoding an immunogenic F or G protein region of one RSV  
3 subgroup virus is incorporated within a genome or antigenome of  
4 a different RSV subgroup virus.

1 21. The RSV of claim 11, wherein the RSV genome or  
2 antigenome is modified to encode a non-RSV molecule selected  
3 from a cytokine, a T-helper epitope, a restriction site marker,  
4 or a protein of a microbial pathogen capable of eliciting a  
5 protective immune response in a mammalian host.

1 22. The RSV of claim 11, which comprises a gene or  
2 gene from PIV replacing a corresponding gene or gene segment of  
3 RSV.

1 23. The RSV of claim 22, wherein the PIV gene or gene  
2 segment encodes HN or F glycoprotein.

1 24. The RSV of claim 23, wherein the gene segment  
2 encodes a cytoplasmic tail, transmembrane domain or ectodomain  
3 of HN or F of PIV1, PIV2, or PIV3.

1 25. The RSV of claim 11, further comprising a  
2 polynucleotide sequence encoding an immunogenic epitope or  
3 protein region of PIV whereby the RSV elicits an immunogenic  
4 response to both PIV and RSV antigens.

1 26. An isolated infectious RSV particle which  
2 comprises a recombinant RSV genome or antigenome, a major  
3 nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a

4 large (L) polymerase protein, and a RNA polymerase elongation  
5 factor, wherein the genome or antigenome is modified to ablate  
6 or modulate expression of a SH, NS1, NS2 or G gene or a  
7 cis-acting regulatory sequence.

1 27. The RSV of claim 26, wherein the SH gene is  
2 deleted.

1 28. The RSV of claim 26, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1 29. The RSV of claim 26, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1 30. The RSV of claim 29, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene or of PIV.

1 31. The RSV of claim 26, wherein the RSV genome or  
2 antigenome further comprises a gene or gene segment encoding an  
3 immunogenic F or G protein region of a different RSV subgroup.

1 32. The RSV of claim 26, wherein the genome or  
2 antigenome comprises a chimera of a human RSV sequence and at  
3 least one non-human RSV sequence.

1 33. The RSV of claim 26, wherein the genome or  
2 antigenome encodes a human RSV in which a selected gene or gene  
3 segment is replaced with a counterpart gene or gene segment from  
4 a heterologous RSV.

1 34. The RSV of claim 33, wherein the selected gene is  
2 NS1 or NS2 and the counterpart gene is N.

1 35. The RSV of claim 26, wherein the RSV genome or  
2 antigenome further comprises a nucleotide sequence of a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a

4 restriction site marker, or a protein of a microbial pathogen  
5 capable of eliciting a protective immune response in a mammalian  
6 host.

1 36. The RSV of claim 26, which further comprises a  
2 gene or gene from PIV replacing a corresponding gene or gene  
3 segment of RSV.

1 37. The RSV of claim 36, wherein the PIV gene or gene  
2 segment encodes HN or F glycoprotein of PIV1, PIV2, or PIV3.

1 38. An isolated infectious RSV particle which  
2 comprises a recombinant RSV genome or antigenome, a major  
3 nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a  
4 large (L) polymerase protein, and a RNA polymerase elongation  
5 factor, wherein the RSV genome or antigenome is modified by a  
6 termination codon introduced within a selected gene, or by a  
7 change in sequence, position, or presence of a GS or GE  
8 transcription signal relative to the selected gene.

1 39. A method for stimulating the immune system of an  
2 individual to induce protection against respiratory syncytial  
3 virus, which comprises administering to the individual an  
4 immunologically sufficient amount of the isolated attenuated  
5 recombinant RSV of claim 1, 26 or 38 in a physiologically  
6 acceptable carrier.

1 40. The method of claim 39, administered in a dose of  
2  $10^3$  to  $10^6$  PFU of the attenuated RSV.

1 41. The method of claim 39, wherein the attenuated  
2 RSV is administered to the upper respiratory tract.

1 42. The method of claim 39, wherein the attenuated  
2 RSV is administered by spray, droplet or aerosol.

1 43. The method of claim 39, wherein the attenuated  
2 RSV is administered to an individual seronegative for antibodies

3 to RSV or possessing transplacentally acquired maternal  
4 antibodies to RSV.

1           44. A vaccine to induce protection against RSV, which  
2   comprises an immunologically sufficient amount of the isolated  
3   attenuated recombinant RSV of claim 1, 26 or 38 in a  
4   physiologically acceptable carrier.

1 45. The vaccine of claim 44, formulated in a dose of  
2  $10^3$  to  $10^6$  PFU of the attenuated RSV.

1                   46. The vaccine of claim 44, formulated for  
2                   administration to the upper respiratory tract by spray, droplet  
3                   or aerosol.

1                   47. A composition which comprises an expression  
2                   vector comprising an isolated polynucleotide molecule encoding a  
3                   RSV genome or antigenome having at least two attenuating  
4                   mutations, at least one of the mutations specifying a  
5                   temperature-sensitive substitution at amino acid Phe<sub>521</sub>, Gln<sub>831</sub>,  
6                   Met<sub>1169</sub>, or Tyr<sub>1321</sub> in the RSV polymerase gene or a temperature-  
7                   sensitive nucleotide substitution in the gene-start sequence of  
8                   gene M2, and one or more expression vector which comprises one  
9                   or more polynucleotide molecules encoding N, P, L and RNA  
10                  polymerase elongation factor proteins of RSV, whereby upon  
11                  expression an infectious RSV particle is produced.

1                   48. The composition of claim 47, wherein the  
2 infectious RSV particle is a virus.

1                   49. A method for producing an infectious attenuated  
2                   RSV particle from one or more isolated polynucleotide molecules  
3                   encoding said RSV, comprising:

4 coexpressing in a cell or cell-free lysate the  
5 expression vectors of claim 47, thereby producing said  
6 infectious RSV particle.

1           50. The method of claim 49, wherein the RSV genome or  
2 antigenome and the N, P, L and RNA polymerase elongation factor  
3 proteins are expressed by two or more different expression  
4 vectors.

1           51. The method of claim 49, wherein the RSV wherein  
2 the genome or antigenome is modified to ablate or modulate  
3 expression of a SH, NS1, NS2 or G gene or a cis-acting  
4 regulatory sequence.

1           52. The method of claim 51, wherein the SH gene is  
2 deleted.

1           53. The method of claim 51, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1           54. The method of claim 51, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1           55. The method of claim 54, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene or of PIV.

1           56. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a gene or gene segment encoding an  
3 immunogenic F or G protein region of a different RSV subgroup.

1           57. The method of claim 51, wherein the genome or  
2 antigenome comprises a chimera of a human RSV sequence and at  
3 least one non-human RSV sequence.

1           58. The method of claim 51, wherein the genome or  
2 antigenome encodes a human RSV in which a selected gene or gene  
3 segment is replaced with a counterpart gene or gene segment from  
4 a heterologous RSV.

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1       59. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a nucleotide sequence of a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a  
4 restriction site marker, or a protein of a microbial pathogen  
5 capable of eliciting a protective immune response in a mammalian  
6 host.

1       60. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a PIV gene or gene segment encoding  
3 HN or F glycoprotein of PIV1, PIV2, or PIV3.

1       61. An RSV strain selected from *cpts* RSV 248 (ATCC VR  
2 2450), *cpts* 248/404 (ATCC VR 2454), *cpts* 248/955 (ATCC VR 2453),  
3 *cpts* RSV 530 (ATCC VR 2452), *cpts* 530/1009 (ATCC VR 2451), or  
4 *cpts* 530/1030 (ATCC VR 2455).

1       62. An RSV strain selected from B-1 cp52/2B5 (ATCC VR  
2 2542) or B-1 cp-23 (ATCC VR).

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